December 1993

What Does Biotechnology Have to Offer Beef Cattle Reproduction? Costs and Practical Value

George E. Seidel Jr.
Colorado State University, Fort Collins, CO

Proceedings, The Range Beef Cow Symposium XIII
December 6, 7, & 8, 1993, Cheyenne, WY

What Does Biotechnology Have to Offer Beef Cattle Reproduction? Costs and Practical Value

George E. Seidel, Jr.
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, CO 80523

INTRODUCTION

The term "reproductive biotechnology" has different meanings in different circumstances. I will use a broad definition that ranges from estrus synchronization to DNA manipulation. By far the most relevant and important reproductive biotechnology for beef cattle production is artificial insemination. It is shocking that only around 5% of calves of beef cattle breeds in this country are sired via this biotechnology, but I will not discuss artificial insemination further because the methodology, costs, and applications are well known. In my opinion, the second most important reproductive biotechnology is estrous cycle synchronization; I'll not cover this further because there will be an entire paper devoted to it in this symposium. An important third reproductive biotechnology, ultrasound, also will be covered by another speaker.

Over the next decade, all other reproductive technologies added together will not sum up to the importance of artificial insemination plus estrus synchronization. In fact, most other reproductive biotechnologies currently are completely inappropriate for most beef cattle operations. On the other hand, there will be profitable but specialized niches in some operations for application of nearly all of the biotechnologies that I will describe.

EMBRYO TRANSFER

Probably 45,000 to 50,000 calves of beef cattle breeds are born as a result of embryo transfer each year in the United States, or about 1 out of every 700 to 800 calves. The main application is to amplify the reproductive rates of valuable cows so offspring can be sold or used for breeding purposes. Other applications include changing from grade to registered herds, circumventing infertility, exporting embryos, and genetic testing. This technology is integral for the use of most other biotechnologies to be described later.

Variability of embryo production from individual donors is extreme; with good management, about 25% of donors produce no pregnancies; the average embryo production per donor is about 6 useable embryos, and the mean number of pregnancies per donor is just over 3 (Table 1). However, many assumptions are associated with such data. With excellent management and use of heifers as recipients, pregnancy rates may exceed 70% with unfrozen embryos. However, for a variety of reasons, pregnancy rates frequently are less than 50% per embryo transferred.

Table 1. Distribution of Numbers of Pregnancies from 64 Superovulated Donors

No. pregnancies	No. donors	Percentage of donors	Percentage of pregnancies
0	14	22	0
1-2	16	25	11
3-4	16	25	26
5-7	11	18	31
8-10	4	7	16
11+	3	5	15

Successful embryo transfer programs require attention to detail, and usually cost some hundreds to more than $1,000 per calf produced, over and above conventional costs of producing calves. With the right market, this can be very profitable, but if conditions are inappropriate, it is easy to lose money. It is highly recommended that a successful artificial insemination program be in place (including personnel, feed, facilities, and cattle) before embryo transfer is attempted.

CRYOPRESERVATION OF EMBRYOS

This biotechnology has become an integral part of embryo transfer technology because it increases flexibility enormously. It takes somewhat more than 1 hour to freeze a batch of embryos, and around 20 minutes to thaw embryos and remove cryoprotectants. The main use is in freezing excess embryos when more are produced than the number of recipients available at the right stage of the estrous cycle; conversely, embryos can be thawed when there is an excess of available recipients. In fact, one can decouple the embryo collection and embryo transfer processes entirely with this biotechnology. This is especially useful when donors are not at the desired stage of the annual reproductive cycle (usually 60-90 days post-partum) when the potential recipients are. There also are the obvious options of using distant donors, even importing and exporting embryos. There is a special advantage to moving germ plasm via embryos because the danger of spreading disease is much lower than with animals or semen. Special precautions such as extra rinsing are taken when freezing embryos for export purposes, and these depend on the importing country, so plans must be made beforehand. Whether for domestic or export sale, embryos represent an additional commodity for a beef stock producer. In some countries, frozen embryos provide a practical method for twin production. In most cases, embryo transfer procedures are too expensive for this to be profitable in the United States.

The major cost of freezing embryos is that pregnancy rates are reduced 10-15% with high quality embryos, and are still lower with embryos of marginal quality; thus, the latter type rarely are frozen. These costs are, however, recouped due to lower recipient needs, which reduces feed and management costs. In many cases, the most profitable strategy is to have recipients available for most embryos and to freeze the excess, thereby taking advantage of the higher pregnancy rates with fresh embryos, and saving costs of preparing recipients that will not be used if embryo production is low. A minor cost of frozen embryos is the freezing and thawing process itself.

This plus costs of equipment, liquid nitrogen, etc. is $10 to $50 per embryo under most circumstances. Over half of the embryos collected in this country are frozen, which illustrates the current utility of this technology.

BISECTION OF EMBRYOS

Good to excellent quality embryos can literally be cut in half with microtools under a microscope. If both halves develop, identical twins are produced. With good management and personnel appropriately trained in microsurgery, the pregnancy rate per half embryo is around 50%. Since two halves are produced, the net pregnancy rate would be 100% per original embryo. Usually each half embryo is transferred to a different recipient to avoid problems that sometimes occur with twin pregnancies. Of course, the two halves can be placed in the same recipient without danger of producing freemartins since both halves will be of the same sex. With a 50% pregnancy rate per half embryo, identical twins occur for ¼ of the embryos, single pregnancies for ½ of the embryos, and no pregnancy for ¼ of the embryos. Success rates decrease markedly when embryos are divided into 3 or more pieces.

This technique is especially valuable when only one or two embryos are recovered from a valuable donor. Thousands of calves have been produced with these methods. There are no more abnormalities with calves resulting from bisected embryos than from artificial insemination without embryo transfer. Even though these procedures work, probably fewer than 1,000 calves per year result from this procedure. The reasons that this is not used more seem to be: 1) Only a few embryo transfer practitioners have the training and equipment for such work; 2) bisected embryos have low pregnancy rates after freezing; and 3) the procedures take 20-30 minutes per embryo (with setup time) and thus disrupt the flow of work. The costs are under $50 per embryo bisected under most circumstances.

SEXING EMBRYOS

There are several methods of sexing embryos, and one is available commercially. This technique requires removal of a few cells of the embryo, using techniques similar to those for bisecting embryos. The genetic material (DNA) is extracted from these cells, and millions of copies of a small segment of DNA on the Y-chromosome (if there is a Y-chromosome) are produced by a technique called the polymerase chain reaction. A test then determines whether this part of the Y-chromosome is present (male) or absent (female). The whole procedure takes several hours. About 85% of bovine embryos are sexable with this approach in skilled hands, and the accuracy is over 95%. Thus, one gets about 43% males, 42% females, and 15% of unknown sex, with few mistakes for those that are designated males or females.

Surprisingly, this technology has not really caught on. Only a few thousand embryos are sexed annually in the United States. This may be due to the lack of trained people and the extra time and expense required. The cost of sexing often is around $100/embryo, but if only one sex is worth transferring, the cost is closer to $400 extra per pregnancy because half of the embryos would be discarded, and just over half of the remainder would develop to term.

For most beef cattle donors, calves of both sexes are valuable, so there is little point in sexing because all embryos would be transferred anyway. If the objective is just to get information about sex, this can be done more easily and cheaply with ultrasound at about 60 days of gestation. In some cases, costs of embryo transfer would be justified by calves of one sex, but not the other. But at $400 extra per correctly sexed pregnancy, this approach must be examined carefully. Sexing embryos will be used more and more as this technology becomes simpler and less expensive. Eventually, however, it will be replaced entirely with sexed semen.

SEXING SEMEN

There is no commercially available method for sexing semen, but there is a method that works under laboratory conditions. X-chromosome-bearing bull sperm (which lead to females) have about 4% more DNA than Y-chromosome-bearing ones (which lead to males). By placing sperm in a solution of DNA-binding dye, X-chromosome-bearing sperm become more brightly stained than Y-chromosome-bearing sperm. By use of lasers plus a device called a cell sorter, it is possible to separate the sperm into three test tubes, male, female, and unsexable (the great majority). Those in the sexed test tubes accumulate at about 1,000 sperm per second, with an accuracy of just over 80%.

Although 500 sperm per second of each sex is phenomenally rapid, in reality it is too slow to be practical for most purposes. For example, one straw of semen often contains 50 million sperm (30 million motile before freezing). At 500 sperm per second, it would take 100,000 seconds per straw or more than 24 hours to produce 1 dose of semen. The logistics of keeping sperm viable for such lengths of time at room temperature are considerable. The equipment is very expensive (over $50,000 per setup) and complicated to operate. Fertility probably would be lowered somewhat, and the long-term consequences of adding dye to DNA are unknown. However, procedures for sexing sperm likely will improve markedly over the next few years, and one practical application, use of such sperm with in vitro fertilization (which requires few sperm), may not be far off.

IN VITRO FERTILIZATION

In vitro fertilization consists of four steps: 1) preparing the sperm (termed capacitation), 2) maturing the unfertilized eggs (oocytes), 3) placing the sperm and oocytes together for fertilization, and 4) keeping the embryos in an incubator for 5-6 additional days while they develop through 2-cell, 4-cell stages, etc. and become suitable for freezing or embryo transfer. I will not explain the technical details of these steps, but point out that they require great attention to detail for over 1 week, and cannot be done practically on the farm.

The main application of in vitro fertilization is to circumvent infertility, much as it is used in women. A related application is to obtain more pregnancies than otherwise possible in cows that do not respond to superovulation. A third application is to obtain calves by recovering oocytes from cows near death or even a few hours after death. In fact, for experimental purposes, we routinely obtain ovaries from slaughterhouses and recover the oocytes up to 6 hours after slaughter.

Oocytes are recovered from living cows by aspirating follicles on the ovaries through a long needle inserted into the body cavity through the wall of the vagina. The needle is guided precisely by using an ultrasound machine to visualize the needle and the ovary. The donor cow need not be injected with hormones to stimulate follicular growth; the oocytes are matured in vitro anyway. All follicles readily visualized (usually those 3 mm or more in diameter) are aspirated, generally resulting in 10 or more oocytes per pair of ovaries. Usually, nearly half are abnormal and discarded. Typically 75% of oocytes become fertilized, 35% of these develop normally to the late morula or blastocyst stage, and pregnancy rates are 40%. Multiplying 50% normal x 75% fertilized x 35% developing normally x 40% pregnancy yields a net pregnancy rate of 4.9% per oocyte aspirated, or about ½ pregnancy per try. However, this can be repeated weekly, which results in an average of two pregnancies per month. This has been repeated with some cows for more than 1 year, though typically breeders stop after 10-15 pregnancies.

Five or six companies with reasonably documented success offer this service in the United States. Typically they charge $500 per pregnancy more than with conventional embryo transfer procedures. Usually three technicians are required to make this work well. Probably over 1,000 calves will be produced by these methods in 1993. In the future, in vitro fertilization will be combined with sexed semen to produce sexed pregnancies.

CLONING BY NUCLEAR TRANSPLANTATION

The objective of this technology is to produce large numbers of genetically identical animals (clones). Most of the cells in a 32-cell (or earlier stage) embryo contain a totipotent nucleus, which means that if the nucleus of one of these cells is transplanted to an oocyte, a genetic copy results that can result in a calf (32 identical calves if all nuclei are used and everything goes perfectly). As the embryo develops to more cells, most cells become so specialized that they are no longer totipotent, and therefore, will not produce a calf. Probably 20-30 cells remain totipotent, even in a 200-cell embryo. Eventually most (all?) of these cells probably lose totipotency. With this technique, one is essentially fertilizing an oocyte with a diploid nucleus (one that already has male and female genetic components) rather than a haploid sperm. With nuclear transplantation, the female genetic material in the oocyte is removed first because the nucleus from the embryo already has both male and female genes. With this technology, one can make clonal copies of embryos, but not adults.

Unfortunately, only pedigree information is available concerning performance of the resulting calves before they are born. Although the set of calves will be genetically identical with each other, it is possible that they all will be mediocre phenotypically due to the vagaries of animal breeding. One solution to this problem is to freeze some of the embryos produced, and allow only a few to develop into calves initially. If they turn out to be excellent animals, genetic copies can be made from the frozen identicals. One can theoretically make an infinite number of copies by serial nuclear transplantation, that is when the copies reach the 32-cell stage the whole set of nuclei can be retransplanted into oocytes.

This technology currently is not available commercially, even though over 1,000 calves have been produced. There are two major problems. The first is that success rates are low.

Theoretically, one could get 32 embryos developing from transplanting nuclei from a 32-cell embryo. In practice, more like 5 or 6 develop on the average. Also, pregnancy rates per developing embryo are low, often around 20%. Thus, one frequently only gets one calf from the whole exercise. Success rates are much higher for some embryos for unknown reasons, and with serial nuclear transplantation, one might get 5 embryos the first round, 25 the second, 125 the third, etc. Even so, the largest set of identicals produced so far is eleven.

The second major problem is that many of the calves are abnormal at birth. They tend to be very slow to get up and nurse, become cold easily, and around 20% are larger than normal. Calves in the 130-140 lb range are common.

The reason for these problems is not known. With excellent veterinary care, including calving in a warm, dry area, and Caesarian section if calves are too big, death losses can be under 10%. Out in the field, half of the cloned calves sometimes die. Interestingly, if they survive, the calves become fairly normal after a few days, and they do not have problems calving themselves or transmit these problems to the next generation. Thus, the problem is not genetic. In the long run, success rates with cloning by nuclear transplantation probably will increase markedly, and the problem with abnormal calves probably will be solved.

DNA-BASED TECHNOLOGIES

A whole spectrum of technologies depends on the ability to distinguish DNA of one animal from that of another. The DNA can be obtained from blood or semen. Broadly speaking, the principles are similar to blood-typing, but DNA typing is much more powerful. One example is detection of deleterious recessive genes (a gene is a short, functional segment of DNA, that usually provides the information for making a protein), such as bovine leucocyte adhesion deficiency (BLAD), in carrier animals. Another example is definitive paternity testing in multiple sire mating pastures. The techniques of marker-assisted selection also are based on DNA typing; the procedure for sexing embryos described earlier is a special case of marker-assisted selection.

A big advantage of DNA typing is being able to determine the genetic make-up of an individual early (even as an embryo), and without breeding trials. Knowing whether an individual is homozygous or heterozygous for traits such as polled, black color, and normal growth (not a dwarf carrier) will be very useful. It is not yet possible to DNA type cattle for all of these characteristics because of incomplete information about the DNA sequence of some of these genes. However, the requisite information is becoming available for more genes each year.

Several companies and university and government laboratories have provided DNA typing services as a part of research and development projects. However, these services have been heavily subsidized. The techniques of DNA typing require fairly technical procedures. Only a few companies and universities provide these services on a routine basis, and only for a few applications. Fees are likely to be in the $10-50 per sample range as this technology matures; tests for additional traits on a particular blood or semen sample likely will be around $5-10 per trait. The key will be volume; 50,000 samples per year will be much more inexpensive

per sample than 5,000 per year. It would seem that large scale paternity testing in multi-sire breeding pastures, for example, would in most cases be difficult to justify if it a costs $10 or more per animal in addition to costs of obtaining the blood.

TRANSGENIC ANIMALS

It is now possible to modify the genetic make-up of animals directly by adding, deleting, or correcting genes in early embryos. For example, one might add the polled trait to a particular breed or line without changing other genes. The DNA for transgenic procedures can even come from other species. For example, a growth gene might be added from a chicken, or a disease resistance gene from an alligator. While a handful of transgenic cattle have been made, this biotechnology is so expensive and impractical that it may be 10 to 20 years before it is used directly in beef cattle production. However, transgenic procedures are very useful for certain kinds of research, and the information from this research likely will be applied within the next few years.

ADDITIONAL READING

The following books provide additional details about the biotechnologies discussed. These currently are available for a small fee from the Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO 80523, telephone 303-491-8373.

Elsden, R.P. and G.E. Seidel, Jr. 1990. Procedures for Recovery, Bisection, Freezing, and Transfer of Bovine Embryos. 43 pp.

Seidel, G.E., Jr. and R.P. Elsden. 1988. Embryo Transfer in Dairy Cattle. 97 pp.

Seidel, G.E., Jr. and S.M. Seidel. 1991. Training Manual for Embryo Transfer in Cattle. 164 pp.